UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
CURSO DE GRADUAÇÃO EM BIOMEDICINA

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ÁCIDOS GRAXOS DE CADEIA CURTA E SEU PAPEL NA PATOFISIOLOGIA DO HOSPEDEIRO

Porto Alegre
2021

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

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Porto Alegre

2021

CIP - Catalogação na Publicação

Behrens, Luiza
Ácidos Graxos de Cadeia Curta e seu papel na Patofisiologia do Hospedeiro / Luiza Behrens. -- 2021.
59 f.
Orientador: José Cláudio Fonseca Moreira.

Coorientador: Lucas dos Santos.

Trabalho de conclusão de curso (Graduação) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Curso de Biomedicina, Porto Alegre, BR-RS, 2021.

1. Ácidos Graxos de Cadeia Curta. 2. Microbiota Intestinal. 3. Fisiologia do Hospedeiro. 4. Saúde Humana. 5. Comunicação entre órgãos. I. Moreira, José Cláudio Fonseca, orient. II. dos Santos, Lucas, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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Aprovado em: 27 de abril de 2021.

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AGRADECIMENTOS

De todos os desafios que eu imaginava que iria enfrentar durante a graduação, a pandemia foi o maior e mais inesperado deles. Foi muito difícil tentar manter a produtividade durante esse período, foram muitas mortes que poderiam ser evitadas, muito descaso com a ciência... Mas graças a algumas pessoas pude chegar até aqui, e não poderia deixar de agradecê-las.

Ao professor José Cláudio, agradeço por todo o aprendizado que me proporcionou. Um marco para mim foi o dia que o conheci, numa palestra sobre o eixo cérebro-intestino, da qual saí completamente fascinada e querendo atuar em pesquisas sobre o tema. O procurei e ele me deu a oportunidade de fazer Iniciação Científica o tendo como orientador, e essa oportunidade com certeza mudou o rumo da minha formação como biomédica. Muito obrigada por todas as dicas, conselhos, ensinamentos, puxadas de orelha, por tudo. Serei eternamente grata ao senhor por ter me apresentado o assunto que faz meu olho brilhar.

Graças a essa oportunidade, conheci pessoas incríveis no nosso amado Lab 32. E uma dessas pessoas foi o Lucas, uma das pessoas mais maravilhosas e dedicadas que já conheci. Obrigada por ter me treinado, por todas as horas que passamos juntos tomando um café no laboratório e fazendo experimentos. Você se tornou um grande amigo e quero te levar para toda a vida.

Agradeço também aos demais integrantes do Laboratório 32. Obrigada por terem me recebido, por todo o apoio, pelas conversas, pelos seminários semanais, fofocas e risadas. Adorei conhecer todos vocês e trabalhar com pesquisadores tão incríveis, e espero que nossos caminhos se cruzem muito no futuro.

Por fim, não posso deixar de agradecer aos meus amigos e família, que sempre me apoiaram e incentivaram não só durante a graduação, mas na vida. Obrigada por se preocuparem comigo e por terem me dado todo o suporte necessário para chegar até aqui. Eu não seria quem sou hoje sem vocês, e espero um dia poder retribuir tudo o que já fizeram por mim.

RESUMO

Os ácidos graxos de cadeia curta (AGCC) são metabólitos resultantes da fermentação realizada pela microbiota intestinal e que possuem impacto relevante para a fisiologia do hospedeiro. Os principais AGCC microbianos são o butirato, acetato e propionato, e suas funções são decorrentes da sinalização gerada a partir da ligação com receptores acoplados à proteína G (GPCR) e modulação epigenética de histona deacetilases (HDAC). Os AGCC são absorvidos pelo epitélio intestinal e são utilizados como um importante substrato energético para os colonócitos, com um destaque para o butirato. O que não é consumido pelo epitélio intestinal entra na circulação sanguínea pelos capilares intestinais e migram rumo à veia porta, onde atingem o fígado. O propionato é utilizado como substrato para a gliconeogênese hepática, e dessa forma o acetato se torna o AGCC em maior concentração na circulação sanguínea após passar pelo fígado. Por meio da circulação, os AGCC então atingem o coração, de onde são distribuídos para os tecidos periféricos e para o cérebro, onde o acetato possui capacidade de atravessar a barreira hematoencefálica. A cada parte do organismo no qual os AGCC entram em contato, há a ativação de série de efeitos que são capazes de alterar a fisiologia do hospedeiro, podendo assim estar associados a patologias relacionadas a esses órgãos. Com o rápido desenvolvimento de tecnologias de sequenciamento em massa associadas a análises bioinformáticas mais robustas, pode-se ter maior compreensão acerca da microbiota, seus metabólitos e os mecanismos envolvidos na sua interação com o hospedeiro. Entretanto, novos estudos devem ser feitos para aprofundar o conhecimento acerca dos AGCC e aplicá-lo em prol do benefício humano.

Palavras-chave: Ácidos Graxos de Cadeia Curta. Microbiota Intestinal. Fisiologia do Hospedeiro. Saúde Humana. Comunicação entre órgãos.

ABSTRACT

Short-chain fatty acids (SCFAs) are end products of gut bacteria and are known to impact host physiology significantly. The main microbial SCFA are butyrate, acetate, and propionate. Their functions are due the signaling generated from G protein-coupled receptors (GPCRs) and epigenetic modulation of histone deacetylases (HDACs). SCFAs are absorbed by the intestinal epithelium and are used as a crucial energetic substrate for colonocytes, emphasizing butyrate. SCFAs not consumed by the epithelium go to the bloodstream through intestinal capillaries and migrates towards the portal vein, where they reach the liver. Propionate is known as a substrate for hepatic gluconeogenesis, and then acetate becomes the SCFA find in greater concentrations in the bloodstream after passing through the liver. Through circulation, SCFAs can reach the heart and then are distributed to peripheral tissues and finally to the brain, where acetate can cross the blood-brain barrier (BBB). At each one of these organs, SCFAs can activate a series of effects that can influence the host's physiology and may be associated with pathologies related to these organs. With the accelerated development of mass sequencing technologies in association with bioinformatics analysis, we can understand more and more about the microbiota, its metabolites, and mechanisms involved in microbiota-host interaction. However, much remains to be discovered for SCFAs to be applied for human benefit in novel therapeutic strategies.

Keywords: Short-Chain Fatty Acids. Gut Microbiota. Host Physiology. Human Health. Inter-organ crosstalk.

LISTA DE ABREVIATURAS

AGCC	Ácidos Graxos de Cadeia Curta
DII	Doença Inflamatória Intestinal
DT2	Diabetes do tipo 2
FFAR2	Receptor de ácidos graxos livres 2
FFAR3	Receptor de ácidos graxos livres 3
GLP-1	Peptídeo tipo-glucagon 1
GPCR	Receptor acoplado à proteína G
GPR109A	Receptor acoplado à proteína G 109A
GPR41	Receptor acoplado à proteína G 41
GPR43	Receptor acoplado à proteína G 43
HCAR2	Receptor de ácido hidroxicarboxílico 2
HDAC	Histona deacetilase
HMP	Human Microbiome Project
IL10	Interleucina 10
IMC	Índice de massa corporal
MCT1	Transportador de monocarboxilato 1
Olfr558	Receptor olfatório 558
Olfr78	Receptor olfatório 78
OR51E1	Receptor olfatório da família 51 e subfamília 1
OR51E2	Receptor olfatório da família 51 e subfamília 2
PYY	Peptídeo YY
SMCT1	Transportador de monocarboxilato acoplado à sódio 1
SNC	Sistema Nervoso Central
SNE	Sistema Nervoso Entérico
T2D	Diabetes do tipo 2
Treg	Célula T reguladora
5-HT	Serotonina

LISTA DE FIGURAS

SUMÁRIO

1 INTRODUÇÃO COMPREENSIVA

A frase “todas as doenças começam no intestino”, dita a mais de dois mil anos por Hipócrates, por mais que exagerada que tenha sido, só mais recentemente vem demonstrando o fundo de verdade que carrega. Com o desenvolvimento das tecnologias de sequenciamento associadas a análises bioinformáticas, dados cada vez mais sofisticados estão surgindo e auxiliando na compreensão acerca da microbiota humana, e a sua importância para diversos órgãos do hospedeiro.

Possuímos uma microbiota distinta em quase todos os nichos do nosso corpo, tais como: pele, vias aéreas, trato urogenital e olhos, sendo o trato gastrointestinal o responsável por abrigar a maior parte dos residentes microbianos do nosso organismo. O intestino abriga uma vasta população de fungos, archaeas e vírus, sendo a população bacteriana a mais abundante e que está melhor caracterizada atualmente pela literatura científica (ECKBURG *et al*., 2005).

O trato intestinal humano abriga um ecossistema microbiano complexo e dinâmico, onde há mais de 100 trilhões de micróbios que somam 1-2 kg em um humano adulto (FORSYTHE e KUNZE, 2013), sendo um valor comparável ao peso do cérebro humano adulto (PARENT e CARPENTER, 1996). Além disso, mais de 99% dos genes presentes no nosso corpo são de origem microbiana, de forma que o microbioma humano supera em mais de 150 vezes a quantidade de genes presentes no genoma humano (QIN *et al*., 2010). Com números de tanto destaque quanto esses, fica claro que o ser humano na verdade é um superorganismo e que é necessário estudá-lo levando a microbiota em consideração.

Projetos de sequenciamento microbiano em larga escala como o Human Microbiome Project (HMP) (TURNBAUGH *et al*., 2007; HUMAN MICROBIOME PROJECT CONSORTIUM, 2012), o European MetaHIT (QIN *et al*., 2010) e o projeto Eldermet (CLAESSON *et al*., 2012) contribuíram para identificar a microbiota humana e revelar que esta consiste em pelo menos 40 mil cepas bacterianas de 1800 gêneros diferentes (LUCKEY, 1972; FRANK e PACE, 2008; FORSYTHE e KUNZE, 2013).

A microbiota é composta principalmente por organismos anaeróbicos estritos, que superam o número de bactérias anaeróbicas facultativas e aeróbicas em 2 a 3 vezes, aproximadamente (CLEMENTE *et al*., 2012). Os filos presentes em maior abundância na microbiota são Firmicutes e Bacteroidetes, com menores números de Proteobacteria, Fusobacteria, Cyanobacteria, Verrucomicrobia e Actinobacteria (QIN, 2010). A microbiota intestinal em adultos pode ser dividida entre dois grandes enterotipos de acordo com o tipo de bactéria dominante, os quais estão fortemente associados com a dieta do hospedeiro (WU *et al*,

2011). O enterótipo 1 possui as Bacteroides como população predominante, que são metabolizadoras de proteínas, enquanto o enterótipo 2 contém predominantemente Prevotella.

Embora haja variação interindividual na composição da microbiota, há um conjunto de funções conservadas entre o microbioma dos indivíduos (TURNBAUGH e GORDON, 2009), sugerindo que é a funcionalidade da microbiota que possui maior importância para o hospedeiro, e não a composição em si.

A microbiota pode ser influenciada por diversos fatores ambientais, como a dieta, estilo de vida ou habitat (MARQUES *et al*., 2010), além de infecções, modo de nascimento (parto normal ou cesárea), uso de medicamentos (principalmente antibióticos), estressores ambientais e genética do hospedeiro (ROTHE e BLAUT, 2013; ZHANG *et al.*, 2015; COMPARE *et al*., 2016)

Um dos mecanismos pelo qual a microbiota interfere na saúde e doença humana é a capacidade de produzir metabólitos a partir da dieta e que possuem capacidade de impactar diversos aspectos da fisiologia do hospedeiro. Uma das classes de metabólitos de maior destaque liberados no lúmen intestinal são os Ácidos Graxos de Cadeia Curta, que serão mais detalhados a seguir.

1.1 Os Ácidos Graxos de Cadeia Curta

Um dos principais tipos de metabólitos produzidos pela microbiota intestinal são ácidos graxos orgânicos que possuem entre 2 e 6 moléculas de carbono em sua estrutura, conhecidos como Ácidos Graxos de Cadeia Curta (AGCC). Os principais AGCC sintetizados pela microbiota intestinal são o butirato, acetato e propionato. (CUMMINGS *et al*., 1987).

Em humanos, os AGCC são resultantes da fermentação de carboidratos complexos, fibras e amidos provenientes na dieta e que nossas enzimas digestivas não são capazes de decompor. Essas substâncias passam por um processo de fermentação bacteriana (principalmente ceco e no cólon) e os AGCC são formados em consequência (CUMMINGS *et al*., 1987; MACFARLANE e MACFARLANE, 2012). Entretanto, quando esses substratos estão em escassez na dieta, os micróbios se adaptam e utilizam substratos menos favoráveis como proteínas e gorduras da dieta (CUMMINGS e MARCFARLANE, 1991; WALL *et al*., 2009).

Os gêneros bacterianos presentes na microbiota que possuem maior associação com a produção de AGCC são: *Akkermansia*, *Bacteroides*, *Bifidobacterium*, *Prevotella*,

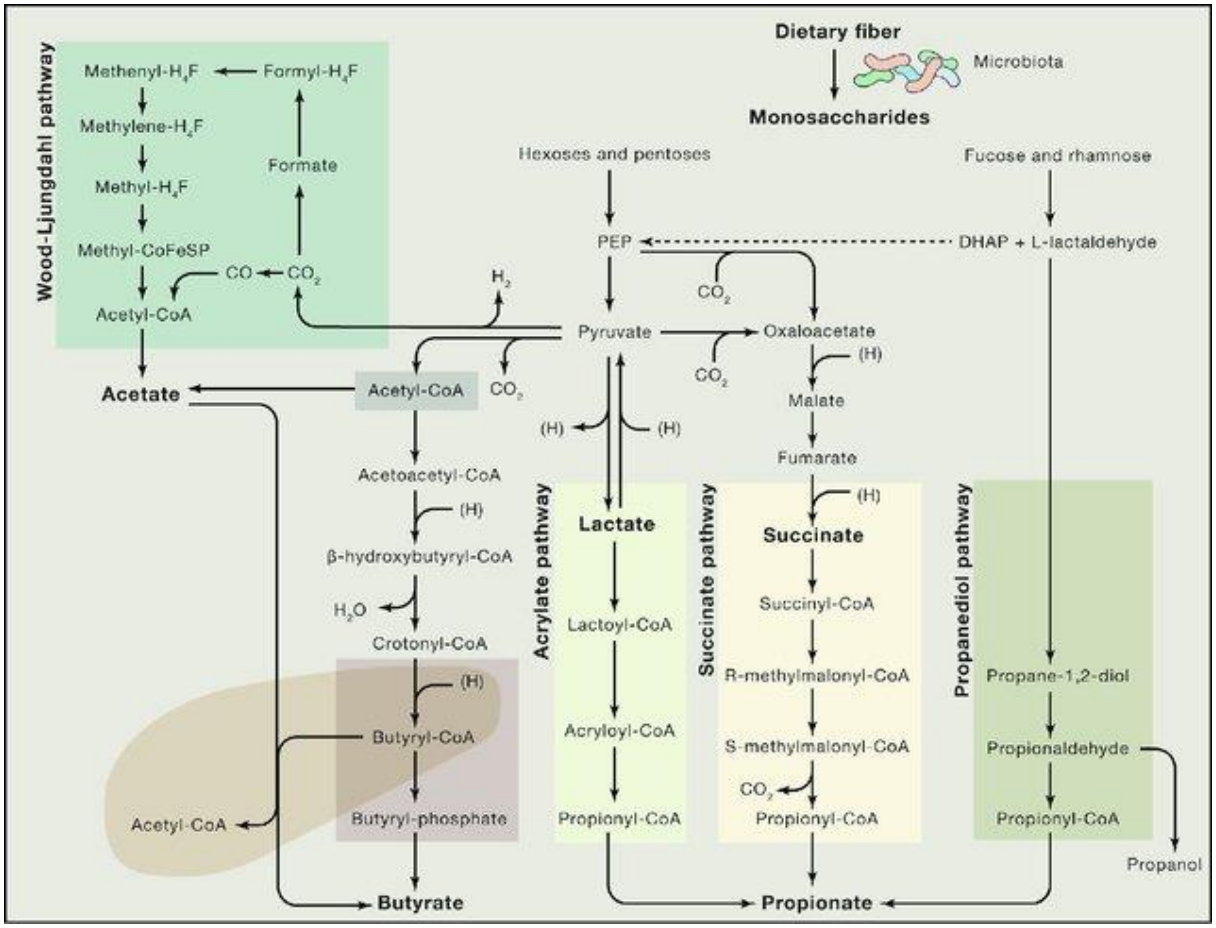
Ruminococcus, Blautia, Chlostridium e *Streptococcus* para o acetato (LOUIS *et al*., 2014; REY *et al.*, 2010). Já para o acetato, são os gêneros *Bacteroides, Phascolarctobacterium, Dalister, Beilonella, Megasphaera, Coprococcus, Salmonella, Roseburia* e *Ruminococcus* (LOUIS *et al*., 2014; SCOTT *et al*., 2006). Por fim, em relação à síntese de butirato, os gêneros são: *Coprococcus, Canaerostripes, Eubacterium, Faecalibacterium* e *Roseburia* (DUNCAN *et al*., 2002; LOUIS *et al*., 2014).

A conversão de substratos provenientes da dieta em AGCC envolve diversas reações mediadas por enzimas bacterianas (Figura 1). O acetato, por exemplo, pode ser produzido a partir do piruvato por várias bactérias intestinais, seja via acetil-CoA ou pela rota Wood-Ljungdahl, na qual o acetato é sintetizado via redução do CO_2 em formato ou via redução de CO_2 para CO seguida da combinação com um grupo metil para produzir acetil-CoA (RAGSDALE e PIERCE, 2008).

Outro importante AGCC, o propionato, é sintetizado a partir da conversão do succinato em metilmalonil-CoA através da via do succinato. O propionato também pode ser sintetizado a partir do acrilato tendo o lactato como um precursor através da via do acrilato (HETZEL *et al*., 2003) e pela via do propanodiol, na qual açúcares como fucose e ramnose servem como substratos (SCOTT *et al*., 2006).

O terceiro principal AGCC, o butirato, é formado a partir da condensação de duas moléculas de acetil-CoA e uma subsequente redução em butiril-CoA, que pode ser convertido em butirato pelas enzimas fosfotransbutirilase e butirato quinase (LOUIS *et al*., 2004). O butiril-CoA também pode ser transfortmado em butirato pela rota butiril-CoA/acetato CoA-transferase (DUNCAN *et al*., 2002). Alguns micróbios também são capazes de utilizar lactato e acetato para a síntese de butirato, o que evita o acúmulo de lactato e estabiliza o meio intestinal. Há análises de metagenoma que sugerem que o butirato também pode ser sintetizado a partir de proteínas através da via da lisina (VITAL *et al*., 2014), sugerindo que os micróbios intestinais podem se adaptar às mudanças nutricionais para manter a síntese de metabólitos essenciais.

Figura 1 – Vias conhecidas de biossíntese dos AGCC



Fonte: Koh *et al*., 2016.

A concentração de AGCC no intestino pode variar dependendo da composição da microbiota, do trânsito intestinal, da interação microbiota-hospedeiro e da dieta do indivíduo. Geralmente essa concentração se encontra na faixa de 20 a 140 mM no lúmen intestinal variando ao longo do comprimento do intestino, possuindo níveis mais elevados no ceco e cólon proximal e havendo diminuição em direção ao cólon distal (CUMMINGS *et al*., 1987), numa proporção aproximada de 60:20:20 para acetato, propionato e butirato, respectivamente (GANAPATHY *et al*., 2013).

Os AGCC estão mostrando cada vez mais o seu papel como peças-chave das interações entre microbiota e hospedeiro, possuindo um relevante impacto na saúde humana e no desenvolvimento de doenças. A seguir, serão descritos os mecanismos de sinalização pelos quais os AGCC agem na fisiologia.

1.2 Receptores de AGCC

Muitas das propriedades regulatórias dos AGCC ocorrem devido às vias de sinalização ativadas por receptores acoplados às proteínas G ou, em inglês, *G protein-coupled receptors* (GPCR), como o GPR43 (também conhecido como *free fatty acid receptor* 2 – FFAR2), GPR41 (ou FFAR3) e GPR109A (também conhecido como *hydroxycarboxylic acid receptor* 2 - HCAR2), moléculas que agem como receptores de AGCC no organismo (ROOKS e GARRETT, 2016). Existem também os receptores olfatórios da família 51 e subfamília 1 (OR51E1) e 2 (OR51E2), mas com menos dados na literatura. Uma representação dos receptores de AGCC pode ser vista na Figura 2.

Os GPCRs que se ligam aos AGCC são expressos não só em enterócitos intestinais, mas também em outros tipos celulares de outros órgãos e tecidos, como fígado, musculatura, neurônios entéricos e também em células do sistema imunológico, sendo um forte indício da amplitude de seus efeitos no organismo.

1.2.1 FFAR2/GPR43

O GPR43, também conhecido como FFAR2, foi identificado como um receptor de AGCC ativado principalmente por acetato e propionato, seguido por butirato (BROWN *et al*., 2003; LE POUL *et al*., 2003). FFAR2 é expresso pelas células L intestinais, onde estimulam a liberação de peptídeo YY (PYY) e peptídeo semelhante a glucagon 1 (*glucagon-like peptide-1*, GLP-1) quando ativado. Em consequência da liberação de GLP-1 no intestino, o acúmulo de gordura nos tecidos adiposos é suprimido, levando ao aumento da sensibilidade à insulina (TAZOE *et al*., 2009). FFAR2 também está expresso em tecidos imunes, havendo evidências de que a microbiota intestinal e FFAR2 regulam a resposta inflamatória na colite (MASLOWSKI *et al*., 2009). Como a resposta inflamatória também está relacionada com o desenvolvimento de obesidade e de diabetes do tipo 2 (DT2), a regulação da função imune via FFAR2 pode também estar relacionada com efeitos metabólicos benéficos dos AGCC.

1.2.2 FFAR3/GPR41

O GPR41 (ou FFAR3) também foi identificado como um receptor de AGCC. Esse receptor é ativado principalmente por propionato e butirato (BROWN *et al*., 2003; LE POUL *et al*., 2003) e também está expresso em células L secretoras de PYY e de GLP-1, indicando

seu envolvimento na homeostase energética (TAZOE *et al*., 2009). FFAR3 também está expresso abundantemente nos gânglios neuronais simpáticos, em particular o gânglio cervical superior, que é responsável por controlar o gasto de energia por meio de efeitos neurais e hormonais sobre o metabolismo da glicose e gordura (KIMURA *et al.*, 2011). A ativação de FFAR3 pela interação com o propionato gera um aumento no ritmo cardíaco e no gasto de energia através da ativação simpática, além de levar à liberação de noradrenalina pelos neurônios simpáticos (INOUE *et al*., 2012). Logo, há indícios de que FFAR3 regula a atividade simpática ao detectar o estado nutricional do indivíduo, mantendo assim a homeostase da energia corporal.

Também há evidência de que FFAR3 contribui para uma melhora da resistência à insulina pelas fibras dietéticas através a ativação do FFAR3 expresso nos nervos periféricos por meio dos AGCC produzidos pela microbiota (CUMMINGS *et al*., 1987). Isso implica que o estímulo de FFAR3 gerado por AGCC exibe efeitos benéficos no metabolismo do hospedeiro por meio do sistema nervoso periférico e da secreção de hormônios no intestino. Assim como FFAR2, FFAR3 também afeta a resposta inflamatória. O propionato se mostrou capaz de afetar a hematopoiese da medula óssea de uma maneira dependente do FFAR3 em camundongos induzindo uma maior produção de macrófagos e precursores de células dendríticas, influenciando assim uma resposta inflamatória alérgica (AHMED *et al*., 2009). Dessa forma, FFAR3 pode estar envolvido em efeitos benéficos dos AGCC no metabolismo do hospedeiro através da regulação de respostas imunes.

1.2.3 GPR109A/HCAR2

O GPR109A (ou HCAR2) foi primeiro identificado como um receptor para niacina, mas também é ativado por β-hidroxibutirato e butirato, porém não por acetato e propionato (AHMED *et al.*, 2009). O GPR109A está expresso nas células epiteliais do cólon e sua ativação por butirato é capaz de suprimir a inflamação no cólon e o processo de carcinogênese, promovendo propriedades anti-inflamatórias em macrófagos do cólon e células dendríticas, que induzem a diferenciação de células T reguladoras (Treg) e produtoras de interleucina 10 (IL10) (SINGH *et al*., 2014). Além disso, o GPR109A também está expresso em tecidos adiposos e em macrófagos desses tecidos, tendo um papel na regulação da homeostase lipídica (AHMED *et al.*, 2009).

1.2.4 OR51E1 e OR51E2

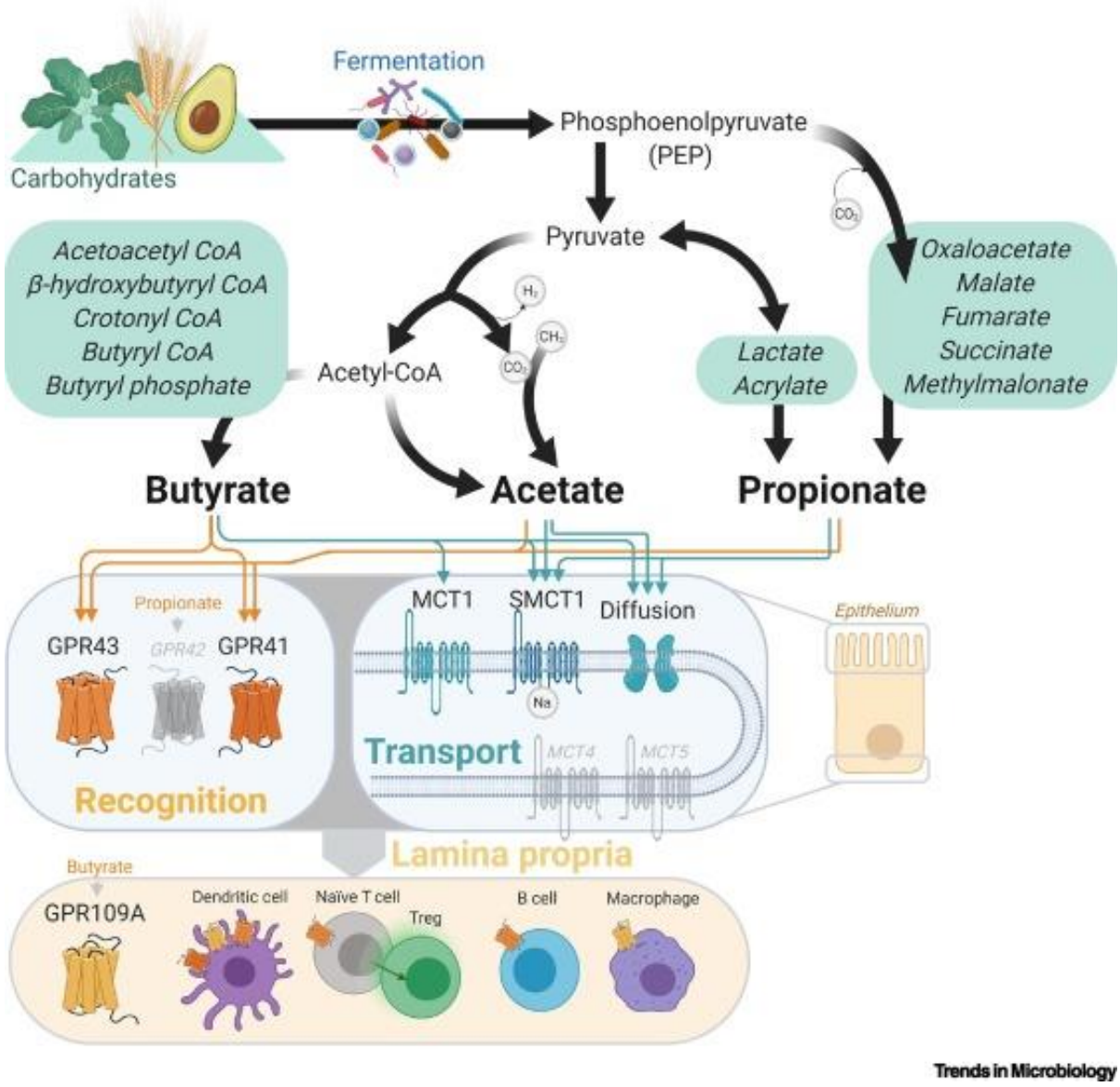
O receptor OR51E1 é equivalente ao receptor olfatório 558 (Olfr558) presente em camundongos, e ambos são suscetíveis a ativação por meio do butirato. Entretanto, são necessárias concentrações mais altas para que a ativação ocorra, sendo improvável que o butirato circulante seja capaz de ativar esses receptores em tecidos além dos intestinais e hepáticos (PRIORI *et al.*, 2015).

Há também o OR51E2, o qual é ativado por acetato e propionato e que também possui um correspondente em camundongos, o Olfr78 (PLUZNICK *et al*., 2013). Ele está expresso em melanócitos humanos (GELIS *et al*., 2016); entretanto, ainda é necessário obter mais dados acerca da contribuição desses receptores para a fisiologia do hospedeiro.

1.3 Transporte de AGCC

AGCC podem ser absorvidos pelo epitélio intestinal de forma passiva mas a maior parte do transporte é realizado de forma ativa via transportador de monocarboxilato 1 (MCT1), e em menor quantidade pelo transportador de monocarboxilato acoplado à sódio 1 (SMCT1). (HALESTRAP e MEREDITH, 2004) Os AGCC, principalmente o butirato, após absorvidos podem ser metabolizados pelos colonócitos, servindo como uma rica fonte de energia (CLAUSEN e MORTENSEN, 1995). O que não for utilizado pelo epitélio é transportado pela membrana celular basolateral através de um mecanismo ainda não bem elucidado, que talvez seja realizado pelo MCT4 ou 5 (DEN BESTEN *et al*., 2013). Na mucosa, os AGCC entram nos capilares sanguíneos e são direcionados para o fígado pela veia porta. O fígado utiliza o propionato como substrato para a gliconeogênese e o acetato acaba se tornando o AGCC mais presente na circulação sanguínea após a passagem do sangue pelo fígado. (BLOEMEN *et al.*, 2009). Os mecanismos de transporte dos AGCC estão representados na Figura 2.

Figura 2 – Esquema representativo dos receptores e transportadores de AGCC



Fonte: Hee e Wells, 2021.

1.4 Regulação Epigenética dos AGCC

Os AGCC produzidos pela microbiota intestinal são capazes de induzir efeitos inibitórios na histona deacetilases (HDAC), funcionando como inibidores não competitivos de HDACs, e foi demonstrado que o butirato e o propionato inibem seletivamente HDAC1 e HDAC3. O butirato é considerado o inibidor mais potente, inibindo principalmente as HDAC das classes I e IIa (CLEOPHAS *et al*., 2016; DAVIE, 2003).

Com relação à influência da inibição de HDAC pelos AGCC nas funções fisiológicas do hospedeiro, foi relatado que o propionato e o butirato produzidos por microorganismos

intestinais promovem a geração de células T regulatórias periféricas (ARPAIA *et al*., 2013; FURUSAWA *et al.*, 2013). O butirato induz a diferenciação de células T reguladoras do cólon, aumentando a acetilação da histona H3 e reduzindo o desenvolvimento de colite (FURUSAWA *et al*., 2013). Além disso, há evidência de que a diversidade da microbiota intestinal e o grau de metilação da região promotora do gene FFAR3 foram significativamente menores nos pacientes obesos e com diabetes do tipo 2 em comparação com indivíduos magros, demonstrando a existência de uma correlação entre o índice de massa corporal (IMC) mais alto e menor metilação de FFAR3 (REMELY *et al*., 2014). Portanto, a regulação epigenética também pode estar relacionada aos efeitos benéficos dos AGCC no metabolismo do hospedeiro.

Recentemente, os AGCC foram associados à crotonilação de histonas, mas a relevância dessa alteração para a fisiologia do hospedeiro ainda precisa ser melhor investigada (FELLOWS *et al.*, 2018).

1.4 AGCC e sinalização endócrina

Os AGCC são capazes de modular o sistema endócrino do hospedeiro por meio do estímulo à liberação de hormônios. Por exemplo, o butirato e o propionato estimulam a liberação do peptídeo tipo-glucagon 1 (glucagon-like peptide 1 – GLP1) e do hormônio intestinal peptídeo YY (PYY) regulador do apetite em células enteroendócrinas intestinais por meio de um mecanismo dependente de FFAR2 (PSICHAS *et al.*, 2015; TOLHURST *et al*., 2012; KAJI *et al*., 2014; CHAMBERS *et al*., 2015). E ainda mais, bactérias produtoras de butirato foram associadas com uma maior secreção de GLP-1 e à maior expressão de genes envolvidos em sua síntese e excreção (YADAV *et al*., 2013). Esses dados sugerem um papel dos AGCC derivados da microbiota intestinal na produção de hormônios endócrinos.

E, além disso, as células enteroendócrinas também são capazes de liberar GLP-2 em resposta à nutrição parenteral com butirato, aumentando as concentrações plasmáticas desse peptídeo. O GLP-2 é capaz de aumentar a área de superfície do epitélio do intestino delgado, aumentando a proliferação celular e inibindo a apoptose (TAPPENDEN *et al*., 2003).

1.5 AGCC e resposta imune

O sistema imune pode ser afetado pela microbiota pelo fato de que há muitas células imunes localizadas no trato gastrointestinal, o que significa que uma perturbação no equilíbrio do ecossistema intestinal também pode resultar em alterações do sistema imune. Os AGCC produzidos pela microbiota intestinal possuem propriedades anti-inflamatórias e podem modular a resposta imune (van de WOUW *et al*., 2018). No intestino, os AGCC são capazes de influenciar a expressão de marcadores anti-inflamatórios, como a interleucina 10 (IL-10) em macrófagos e células dendríticas intestinais (SINGH *et al*., 2014).

A sinalização de butirato através do receptor GPR109A demonstrou conferir propriedades anti-inflamatórias em macrófagos e células dendríticas presentes no cólon (SINGH *et al*., 2014), sendo importantes para a manutenção da homeostase intestinal (WELLS *et al*., 2011). Em outros estudos, os efeitos anti-inflamatórios do butirato e propionato nessas células mostraram ser independentes de GPCRs, mas dependentes da modulação das HDACs (ARPAIA *et al*., 2013; CHANG *et al*., 2014).

O butirato pode agir nas células imunes através dos GPRs (GPR41 e GPR43), que estão expressos nas células imunes, incluindo células polimorfonucleares, indicando um possível envolvimento desse AGCC na ativação dos leucócitos (MEIJER *et al*., 2010). Além disso, o butirato é um importante regulador negativo de inflamação (MASLOWSKI *et al*., 2009; KARLSSON *et al*., 2012).

1.6 AGCC associado a patologias

A microbiota está constantemente em comunicação com os órgãos e sistemas do organismo do hospedeiro, incluindo a medula óssea (JOSEFSDOTTIR *et al*., 2017), vasculatura (KARBACH *et al*., 2016), rins (EVENEPOEL *et al*., 2017), sistema imune (ROOKS e GARRETT, 2016), sistema nervoso autônomo (BERCIK *et al*., 2011; BRAVO *et al*., 2011) e o cérebro (DINAN e CRYAN, 2017). Essa complexa comunicação contribui para a homeostase e a saúde do hospedeiro, e desbalanços na microbiota (conhecida como disbiose) podem estar associados ao desenvolvimento de doenças. A disbiose possui um papel central em várias doenças crônicas, e atenuá-la pode ser uma estratégia em potencial para o controle dessas patologias (KONTUREK *et al*., 2015).

AGCC geram diversos impactos em vários aspectos da fisiologia do hospedeiro, inclusive na suscetibilidade a doenças (EVANS *et al*., 2013).

A influência dos AGCC na (pato)fisiologia do hospedeiro será abordada de forma mais extensiva no artigo presente neste trabalho.

2 JUSTIFICATIVA

Com o surgimento de tantos dados acerca da microbiota intestinal, se torna necessário organizar o conhecimento já adquirido para identificar as lacunas ainda existentes nas quais devem ser focadas novas pesquisas, buscando uma melhor compreensão sobre os AGCC, seus mecanismos de interação com o hospedeiro e seus efeitos no organismo.

Além disso, é necessário ressaltar o potencial de aplicação dos AGCC em estratégias clínica e terapêutica, visando atenuar o quadro patológico de diversas doenças humanas e contribuir para a busca de uma melhor qualidade de vida para o paciente.

3 OBJETIVOS

3.1 Objetivo geral

Realizar uma revisão compreensiva das evidências já presentes na literatura científica acerca dos ácidos graxos de cadeia curta de origem microbiana e sua influência na patofisiologia do hospedeiro, buscando uma maior compreensão de seus efeitos em escala sistêmica.

3.2 Objetivos específicos

3.2.1 Investigar o papel dos ácidos graxos de cadeia curta e as alterações fisiológicas que ocorrem no organismo em decorrência da sua produção pela microbiota intestinal.

3.2.2 Investigar a associação dos ácidos graxos de cadeia curta com o desenvolvimento de patologias humanas.

3.2.3 Investigar o efeito causado pelos ácidos graxos de cadeia curta com foco em órgãos cujas patologias possuem maior associação com a microbiota intestinal, como intestino, fígado, coração e cérebro.

3.2.4 Verificar possíveis interconexões entre os eixos microbiota-fígado, microbiota-coração e microbiota-cérebro.

3.2.5 Identificar lacunas ainda existentes no conhecimento acerca dos ácidos graxos de cadeia curta e que podem se tornar foco de investigações futuras.

4 ARTIGO CIENTÍFICO

Microbial Short-Chain Fatty Acids and Host Pathophysiology

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Highlights

- Short-chain fatty acids (SCFAs) are speculated to have a critical role in host physiology
- SCFAs might interact with the host via G protein-coupled receptor or histone deacetylases
- SCFAs should be quantified in the systemic circulation and fecal samples to the development of specific biochemical assays
- SCFAs could be used in potential interventional strategies and novel therapeutic methods for clinical practice
- Further investigation is still needed to understand SCFAs mechanisms of action fully

Abstract

Short-chain fatty acids (SCFAs) are end products of gut bacteria and are known to impact host physiology significantly. The main microbial SCFA are butyrate, acetate, and propionate. Their functions are due the signaling generated from G protein-coupled receptors (GPCRs) and epigenetic modulation of histone deacetylases (HDACs). SCFAs are absorbed by the intestinal epithelium and are used as a crucial energetic substrate for colonocytes, emphasizing butyrate. SCFAs not consumed by the epithelium go to the bloodstream through intestinal capillaries and migrates towards the portal vein, where they reach the liver. Propionate is known as a substrate for hepatic gluconeogenesis, and then acetate becomes the SCFA find in greater concentrations in the bloodstream after passing through the liver. Through circulation, SCFAs can reach the heart and then are distributed to peripheral tissues and finally to the brain, where acetate can cross the blood-brain barrier (BBB). At each one of these organs, SCFAs can activate a series of effects that can influence the host's physiology and may be associated with pathologies related to these organs. With the accelerated development of mass sequencing technologies in association with bioinformatics analysis, we can understand more and more

about the microbiota, its metabolites, and mechanisms involved in microbiota-host interaction. However, much remains to be discovered for SCFAs to be applied for human benefit in novel therapeutic strategies.

Keywords: Short-Chain Fatty Acids. Gut microbiota. Host Physiology. Human Health. Interorgan crosstalk.

1 Introduction

The human gastrointestinal tract is home to most of the microbial residents of our body. The intestine is home to a vast bacterial population in a complex and dynamic microbial ecosystem, where more than 100 trillion microbes add up to 1-2 kg in a healthy adult[1]. Therefore, it is clear that the human being is a superorganism and that it is necessary to study it considering the microbiota as well. One of the mechanisms with which the microbiota interferes with human health and disease is through metabolites' production. One of the most prominent classes of metabolites released in the gut are short-chain fatty acids (SCFAs). The most studied SCFAs are butyrate, acetate, and propionate[2]. In humans, SCFAs are resultant of complex carbohydrates, non-digestible fibers and starches from diet. These substances undergo bacterial fermentation (mainly on the cecum and colon) and SCFAs are released as result[2].

SCFAs are increasingly showing their crucial role in the microbiota-host interaction and their impact on human health and disease development. Many of the regulatory properties of SCFAs occur due to signaling pathways activated by G protein-coupled receptors (GPCR) such as GPR43 (also known as free fatty acid receptor 2, FFAR2), GPR41 (or FFAR3), and GPR109A (also known as hydroxycarboxylic acid receptor 2, HCAR2)[3]. There are also olfactory receptors of family 51 and subfamily 1 (OR51E1) and 2 (OR51E2), but there are less data about them. SCFAs can also induce inhibitory effects on histone deacetylases (HDACs), functioning as non-competitive inhibitors and, consequently, modulating the human epigenetic.

This is a comprehensive review of the role of microbial SCFAs on the physiology of the host, and our goal is to expand our view of what is already known about SCFAs mechanisms and how it could be used for human benefit, highlighting the high potential for novel therapeutic strategies based on the microbiota and its metabolites.

2 SCFAs and gut

It is believed that the microbiota can generate benefits in the intestinal barrier through SCFAs, which are an essential source of energy not only for the microbiota but also for cells of the intestinal epithelium itself. Moreover, in addition to being a substrate for energy production, SCFAs may affect several processes in the host organism, including host-microbiota signaling, colon pH, intestinal motility, cell epithelium proliferation, fluid and electrolyte absorption, hormone and cytokines secretion, and the maintenance of barrier function[3].

SCFAs have multiple roles in the maintenance of intestinal homeostasis. As for ion absorption, SCFAs have a significant role in NaCl absorption and electrolyte balance. Butyrate, mainly, stimulates NaCl absorption and inhibits Cl- secretion[4]. Also, SCFAs participate in the control of the balance between proliferation and cell apoptosis on the intestinal epithelium[5], induce antimicrobial peptides secretion[6] and differentiation of regulatory T cells (Treg) such as Th17[7].

Between SCFAs, butyrate is the main energy source for colonocytes and is capable of inducing gut gluconeogenesis. Propionate is also used as substrate in the gut but to a lesser extent. There is evidence that intestinal gluconeogenesis enhances beneficial metabolic effects of butyrate and propionate via signalization for increased insulin sensitivity and glucose tolerance[8].

In addition to being an energy substrate, SCFAs significantly influence in maintaining the integrity of the intestinal barrier. That barrier consists in the intestinal epithelium (which can be more or less permeable according to the expression of tight junctions) and a mucus layer full of antimicrobial peptides secreted by the epithelium itself. There is evidence that SCFAs-producing bacteria protects against infections caused by pathogenic bacteria, indicating that SCFAs plays a prominent role in maintaining the intestinal barrier's integrity, preventing the translocation of harmful bacteria and toxins from the lumen to the host[9]. On the other hand, the barrier must be permeable enough for fluids and nutrients absorption. To achieve both these goals, it is necessary to maintain a peaceful coexistence between microbiota and host. There is evidence that disturbances in this relationship can activate inflammatory processes and increase diseases' risk[10]. Reduction in SCFAs levels, especially butyrate, may influence the barrier dysfunction and facilitate the translocation of toxins and LPS from lumen to the bloodstream, activating inflammatory processes[11]. Thus, it reinforced the hypothesis that mechanisms of control of the intestinal barrier are essential for maintaining health, and that microbiota-related disorders can lead to disease development.

One of the mechanisms for the maintenance of the integrity of the gut barrier is the increase of mucin secretion by the goblet cells of the epithelium in response to SCFAs[12],

increasing the mucus layer that protects the epithelium from direct contact with lumen microorganisms. Butyrate, significantly, can increase the expression of tight junction proteins, such as occludine, claudin, and zonula occludens[13] and reduce intestinal permeability, which prevents translocation of bacteria and toxins through the epithelium[14]. Through these mechanisms, the integrity of the gut barrier is preserved.

Likewise, patients who presents a deficit of butyrate-producing bacteria in the gut also shows a disruption of gut epithelium due to lesions in tight junctions (mainly related to claudin 1 and 2 proteins), which leads to an increase in intestinal permeability. This was seen in patients with inflammatory bowel disease (IBD), indicating that increased permeability may increase the host's susceptibility to inflammatory processes and that butyrate deficiency may be an indirect indicator of intestinal barrier function[13]. However, it would still be necessary to develop specific essays for butyrate evaluation to apply this knowledge in clinical practice.

Studies have shown that butyrate is reduced in CRC patients[15] and can have effects on colorectal carcinoma (CRC) cells, potentially reducing cell growth and inducing apoptosis in cancer cells[16]. One of the mechanisms involved is the inhibition of HDAC, preventing an imbalance in histone acetylation that can lead to genes involved in cell control and apoptosis[17]. Another mechanism would be through the GPR109A receptors of the colon, that are capable of activating the apoptosis in tumor cells[18].

We can also mention the anti-inflammatory role of butyrate by inhibiting NF-kB in colon epithelial cells[19], which may result from HDAC inhibition. NK-kB activity is often dysregulated in CRC patients and inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease. In Crohn's disease, butyrate has been shown to reduce pro-inflammatory cytokines' expression by inhibiting NF-kB[20].

Once produced SCFAs are absorbed by gut colonocytes or are eliminated in feces, which are great biological samples for microbiota analysis and are widely used to measure SCFAs production. The absorbed SCFAs that are not consumed by the gut epithelium as an energy source are transported to blood capillaries, migrates towards the portal vein, and reach the liver[2].

4 SCFAs and liver

The gut-liver axis refers to a bidirectional relationship between the gut, its microbiota, and the liver. This reciprocal interaction occurs through the portal vein, which allows the transport of products derived from gut microbiota (such as SCFAs) to the liver and a hepatic

feedback response by bile and antibodies secretion. There is an interdependence between gut and liver, and disturbances in the intestinal barrier could result in an influx of bacteria and microbial products to the liver, resulting in inflammation processes and even developing certain diseases[21].

There is evidence that SCFAs may play a role as a significant substrate for increasing triglyceride levels in the liver. Propionate is described as an essencial substrate for hepatic gluconeogenesis[8]. On the other hand, butyrate can activate oxidation and thermogenesis of fatty acids by increasing the expression of PGC-1α and phosphorylation of AMPK in liver tissues[22]. Thus, SCFAs promote energy storage and weight gain and may be related to pathological states that affect liver homeostasis.

Human studies comparing non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and healthy subjects show a higher fecal concentration of SCFAs in patients with NAFLD and NASH, concomitantly with an increase in SCFAs-bacterial producers. Furthermore, with the increase in fecal SCFAs and microbial signature in NASH, there is an associated reduction in Treg cells and a higher number of Th17 in peripheral blood, a systemic response observed in NASH. In both obesity and NAFLD, there is evidence of an association between clinical phenotypes and SCFAs attributed to a difference in the amount of individual SCFAs, and each one may have different effects on host metabolism[23].

The microbiome of patients with Type 2 Diabetes mellitus (T2D) shows a reduction in the number of butyrate-producing bacteria[23]. Although the microbial dysbiosis in patients with T2D is moderate compared to the dysbiosis reported in obese patients, a study revealed that patients with T2D exhibit a lower number of butyrate-producing bacteria and more significant numbers of opportunistic pathogens. Enrichment of microbial functions associated with reduced sulfate and increased oxidative stress has also been reported, which is indicative of pathogenicity[23]. This promotes the idea that butyrate-producing bacteria protect the host against diseases. Therefore, it is worth considering butyrate as a potential biomarker for intestinal and hepatic health.

Diseases related to the consumption of alcohol can also be correlated with the microbiota. Liver damage from alcohol consumption is marked by a reduction in butyrate and propionate levels[24]. And higher levels of acetate (possibly because of the metabolism of ethanol in the intestinal lumen but mainly by liver metabolism). The reduction in the amount of butyrate is associated with a weakening of the intestinal tight junctions, related to gut permeability[24].

For being an important substrate for liver metabolism, propionate is present in lower concentrations on peripheral tissues, leaving acetate as the most abundant SCFA in the

peripheral circulation[2]. Acetate, therefore, is the most present SCFA in the bloodstream when it reaches the heart.

5 SCFAs and heart

After passing through the liver, SCFAs follows the bloodstream influx and eventually reach the heart and, from there, they can be distributed to the host's peripherical tissues. The concentration of SCFAs in a healthy adult's peripheral blood is estimated at 100-150 umol/L for acetate, 4-5 umol/L for propionate and 1-3 umol/L for butyrate[2]. The latter two are present in lower concentrations because they are metabolized in the gut and liver before reaching the bloodstream, as has already been shown.

As much as SCFAs reach the heart via circulation, they have a greater capacity to influence the cardiovascular system and its pathologies indirectly by signalization pathways from the gut. It has already been shown that in heart failure, for example, the microbiota's diversity is altered, showing depletion of butyrate-producing bacteria[25]. Moreover, this is inversely associated with high levels of soluble CD25 (alpha subunit of the IL-2 receptor) in plasma, a marker for activation of macrophages and T cells. These findings support the potential of the gut microbiota relevance and the modulation of inflammation through SCFAs production in heart disease.

It is hypothesized that when the intestinal barrier is disrupted, there is a more significant translocation of components from the microbiota to the bloodstream. It generates a systemic inflammatory condition that can have a negative impact on patients with heart failure [26]. Evidence is that bacterial translocation occurs in patients with heart failure because of changes in gastrointestinal tract due to splenic congestion, as well as immunological abnormalities. Therefore, SCFAs are how the gut microbiota is involved with the regulation of blood pressure, especially butyrate[27]. Wilck and collaborators demonstrated that salt-related hypertension was associated with the depletion of of SCFAs-producing bacteria strains, which is related with a lower induction of Th17 cells and hypertension[28].

Pluznick and colleagues have shown that the gut microbiota can produce SCFAs and modulate blood pressure through the interaction of SCFAs with GPCRs. The stimulation of these receptors activates pathways that lead to renin secretion, and dysfunctions in this signaling process may be associated with the development of hypertension[29]. The receptors more associates with the development of hypertension through blood pressure changes are FFAR2, FFAR3, and OR51E2[27'29].

In a pathological atherosclerosis context, it is noteworthy that SCFAs can play a crucial role in innate immunity and inflammatory processes through mechanisms involving HDAC and/or GPCR receptors[30]. *Eubacterium rectale*, a butyrate producer, is present at lower levels in atherosclerosis patients[31]. Researchers also observed lower levels of *Faecalibacterium prausnitzii*, other SCFA producer, in older patients with congestive heart failure[32]. Another group reported that the depletion of *Eubacterium* species and higher levels of soluble CD25 in plasma were associated with heart transplantation cases[33].

Mechanisms by which SCFAs modulate cardiac function and physiology in humans still need to be further investigated. However, the measurement of SCFA levels in peripheral blood can be a potential marker for gut microbiota dysfunctions, the intestinal barrier's permeability, systemic inflammation, and predisposition to diseases, and can be used in clinical trials in the future.

6 SCFAs and brain

One of the communication mechanisms between the brain and microbiota are SCFAs bacterial fermentation products[34]. SCFAs can directly affect the central nervous system (CNS) by acetate present in the bloodstream, which can cross the blood-brain barrier (BBB) and reduce appetite through a central homeostatic mechanism related to the expression profiles of appetite regulating neuropeptides in the hypothalamus via activation of the TCA cycle[35].

There is new evidence demonstrating that the microbiota has critical consequences for the host's physiological processes and affects neurodevelopment, behavior, cognition, and synthesis of neurotransmitters[36]. There are GPR43-dependent effects on the central nervous system, including maturation and function of microglia, activation of macrophages, and the maintenance of their homeostasis[3]. Also, AGCC can interact with neuron cells by stimulating the autonomic and sympathetic nervous system through GPR41[37] and GPR43[38] signaling.

Despite the low concentrations in bloodstream, propionate and butyrate can affect peripheral organs indirectly by activating the hormonal and nervous systems, consequently generating an important signalization through neural circuits to increase insulin sensitivity and activating intestinal gluconeogenesis via gut-brain axis. As result, metabolic benefits in body weight and glucose control are enhanced[8].

The enteric nervous system (ENS) communicates bidirectionally with the brain by several mechanisms in addition to those mentioned above. One of them is through the vagus nerve, which sends sensory signals from gut to the nucleus of the solitary tract (NTS) in CNS.

Vagal afferents express receptors that are activated by SCFAs[39] and activation of this pathway is associated with glucose homeostasis[40]. Butyrate has also been shown to affect ENS and intestinal motility[41].

Besides, it is estimated that 90% of the human body's serotonin (5-HT) is produced by enterochromaffin cells (EC) in the digestive tract[42], and the functioning of these cells is known to be modulated by changes in the gut microbiome. One of the pathways in which microbiome disruption affects 5-HT production is through SCFAs; particularly butyrate and propionate have demonstrated the capacity to influence the synthesis of the enzyme tryptophan hydroxylase (a limiting enzyme for serotonin synthesis), aiding the synthesis of 5-HT by EC cells[43]. Recent studies demonstrate that gut microbiota influences the gut-brain axis and shapes symptoms associated to stress, such as anxiety and pain tolerance[44]. Some data suggests a lower visceral perception induced by butyrate via an increased release of 5-HT[45], which can reduce discomfort in several intestinal diseases.

SCFAs are associated with the pathogenesis of several neurodegenerative diseases [46]. Many psychiatric diseases are associated with inflammation and an exacerbated immune response observed by the high level of cytokines[47]. This can be a result, among other causes, of a lower degree of SCFAs production. The interaction of the gut with environmental risk factors for psychiatric diseases, such as diet and stress (especially in early life) suggests that interventions targeting the gut microbiota could ameliorate psychiatric and neurodegenerative symptoms, enhancing the quality of life of the host.

We can also mention the association of SCFAs with the development of autism [48] and alterations in levels of fecal SCFAs seen in patients with Parkinson's Disease[49]. Some of the mechanisms of SCFAs signaling include the regulation of HDAC that can be part of the regulation of hypothalamus-pituitary-adrenal (HPA) axis, which has high association with stress, major depression and anxiety[50].

If in one hand we already have evidence of the effects od SCFAs on CNS and its correlation with psychiatric and neurodegenerative diseases, on other hand there is still a long path to go to understand in depth the mechanisms underlying this signaling process and how we can use it for human benefit.

7 Conclusions and Prospective

In the last few decades, the pace at which new studies on the human microbiota have emerged has increased significantly, revealing the most diverse pathways in which these

microorganisms are capable of impacting our lives. Currently, it is evident that the microbiota is a determining factor for health and disease, having a pivotal role in the regulation of human physiology, and SCFAs have a remarkable prominence as one of the primary mechanisms in which the host-microbiota interaction occurs.

It is already known the central role of SCFAs for maintenance intestinal epithelium homeostasis and being an essential source of energy and substrate for gluconeogenesis. Then, the SCFAs that are absorbed in the gut enter the bloodstream and migrate towards the portal vein, and reaching the liver, where part of them is also metabolized. From that point forward, SCFAs are transported to other peripheral tissues and can even reach the brain. In this process, SCFAs can influence the host physiology directly or indirectly and could be associated with the development of pathologies (or have a protective role against them).

It is still not entirely clear whether SCFAs induces beneficial effects by itself or if it is a consequence of combining them and other microbial metabolites in specific proportions and exact concentrations. However, the perception that gut microbiota can contribute to human health and the susceptibility to diseases promotes a new and broader perspective on understanding human pathologies.

The presence of SCFAs in venous and arterial blood, in addition to stool samples, allows the possibility for the dosage of these substances to be used as a prediction or diagnosis markers of diseases. However, it is still necessary to understand more about the potential that SCFAs have as biochemical markers.

As we acquire more profound knowledge about the microbiota, SCFAs, and their interactions with the host, we increasingly revealing potential therapeutic targets. Nevertheless, there are many gaps about SCFAs and their mechanisms to be revealed. These gaps should be targeted for future research to have therapeutic and preventive strategies based on microbiota one day. We face the challenge of identifying the exact role of SCFAs in the (patho)physiology of the host and need to acquire more knowledge about its mechanisms to use it in clinical practice.

Acknowledgements

We thank our main affiliation with the Biochemistry Department of Federal University of Rio Grande do Sul and the Center of Studies on Oxidative Stress.

Conflict of interest

The authors declare no conflict of interest.

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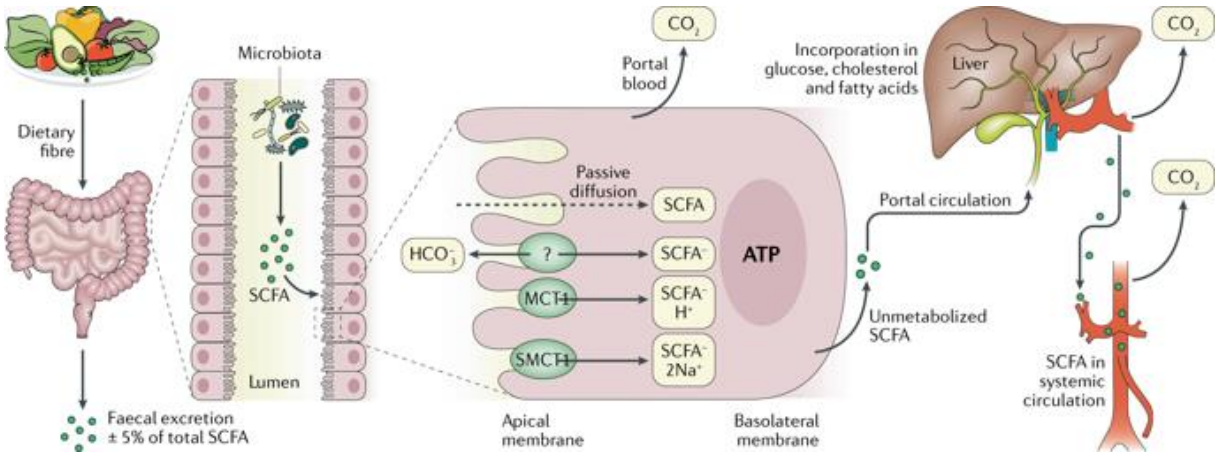
5 CONCLUSÕES E PERSPECTIVAS

Nas últimas décadas o ritmo em que surgem novos estudos sobre a microbiota humana aumentou de forma significativa, revelando as mais diversas formas pelas quais esses micro-organismos são capazes de impactar nossas vidas. Isso só foi obtido graças principalmente a estudos clínicos e translacionais de ponta. Atualmente é evidente que a microbiota é um fator determinante para a saúde e doença do hospedeiro, sendo uma peça-chave para a regulação da fisiologia humana, sendo que os AGCC possuem um grande destaque como um dos mecanismos de interação entre microbiota e hospedeiro.

Já se sabe da grande importância dos AGCC para o epitélio intestinal e manutenção da barreira, além de servirem como fonte de energia e substrato para a gliconeogênese intestinal. Os AGCC que são absorvidos entram na corrente sanguínea e migram em direção à veia porta, atingindo o fígado, onde parte também é metabolizada. A partir desse ponto, os AGCC são distribuídos para os demais tecidos do corpo, atingindo inclusive o cérebro e podendo alterar a fisiologia do hospedeiro de forma direta ou indireta e ter associação com o desenvolvimento de patologias. Um esquema representativo da rota dos AGCC no organismo do hospedeiro pode ser visto na Figura 3.

Figura 3 – Esquema representativo da rota dos AGCC no corpo humano



Fonte: Dalile *et al*., 2019.

Ainda não está totalmente elucidado se os AGCC induzem efeitos benéficos de forma individual ou se seria uma combinação entre eles e outros metabólitos microbianos numa proporção específica, e quais as concentrações exatas para que esses efeitos sejam promovidos.

A percepção de que a microbiota intestinal pode contribuir tanto para a saúde humana quanto para a suscetibilidade de doenças promove uma nova e mais ampla perspectiva sobre o entendimento das doenças.

A presença dos AGCC no sangue venoso e arterial e em amostras de fezes nos permite considerar a possibilidade de que a dosagem dessa substância possa ser utilizada como predição ou diagnóstico de doenças. Entretanto, ainda é necessário um melhor entendimento do seu potencial como marcadores bioquímicos.

Esses, entre outros motivos, fizeram com que eu tivesse um grande interesse no estudo dos AGCC e, no laboratório no qual foi realizado o Estágio em Pesquisa que tornou possível realizar este Trabalho de Conclusão de Curso, me envolvi no desenvolvimento de um projeto que investiga o papel dos AGCC no sistema nervoso. O trabalho teve como foco o estudo da Depressão Maior em modelo animal com alto potencial translacional. Este trabalho está em andamento e possivelmente trará dados muito relevantes para a área.

Ao passo em que vamos adquirindo um conhecimento mais profundo acerca da microbiota, os AGCC e sua interação com o hospedeiro, revelamos novos alvos terapêuticos em potencial. Entretanto, ainda existem muitas lacunas acerca dos AGCC e seus mecanismos de ação, que devem ser alvo de futuras pesquisas e para que um dia o desenvolvimento de estratégias terapêuticas e preventivas baseada na microbiota seja possível.

Temos pela frente o desafio de identificar o papel exato dos AGCC na (pato)fisiologia do hospedeiro e pontuar precisamente os mecanismos pelos quais eles agem: um conhecimento que pode ser extremamente benéfico para a saúde humana e passível de ser utilizado na clínica.

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ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA HUMAN MICROBIOME JOURNAL

For a review article:

Unstructured abstract (up to 250 words)

5-10 keywords

3000 words maximum

3 to 4 tables/figures maximum (optional colour)

50 references maximum

Ensure that the following items are present:

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